Fine structure of oligodendrocytes in the white matter of myelin deficient jimpy mice

J. Shiga Univ. Med. Sci. 3, 1~10, 1988

Fine Structure of Oligodendrocytes in the White Matter of Myelin Deficient Jimpy Mice

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Oligodendrocytes remaining in the white matter of myelin deficient Jimpy mice, sex-linked mutants with a short life of about 25 days, were investigated by electronmicroscopy. In normal control mice, myelination started in the cerebellar medulla on postntal day 3 and in the corpus callosum on postnatal day 7, and it reached adult level by postnatal day 21.

In the Jimpy mutants, glioblasts gave rise to immature oligodendrocytes, but most of them degenerated prior to cell maturation. Thus, there were few myelin forming cells considered to be light oligodendrocytes (LD) at the onset of the myelination period. The mature cells, medium and dark oligodendrocytes (MO, DO) were never found in the white matter of Jimpy mice during this series of experiments. Features suggesting the death of oligodendrocytes were found to be three times more frequent in the white matter of Jimpy mice as compared with that of the controls, especially on postnatal day 12. They usually displayed a denes homogenous nucleus of 1-5μm in diameter and vacuolated cytoplasm filled with fine ribosomal particles and swollen vesicles. A small number of intact oligodendrocytes extended their processes to surround axons. The myelin sheaths in Jimpy white matter were poorly formed. The periodicity of the lamellae was irregular and the thickness of the sheaths was uneven because glial processes wrapping the axons were partially compacted by membrane fusion. The compaction generally occurred in the outer layers rather than in the inner layers. The uncompacted glial processes often displayed fine filamentous and tubular materials.

Key words : Jimpy mutant mouse, oligodendrocyte, white matter, myelination

Introduction

Recently, we have observed alterations in the white matter of Jimpy mice by immunohistochemistry using antibodies against glial fibrillary acidic protein and by electron microscopy (Imamoto, 1985; 1986). The abnormalities in the Jimpy white matter consist of the following: a) low density of glial population, b) scarcity of mature oligodendrocytes, c) hypertrophy and hyperplasia of astrocytes, d) numerous macro-

Accepted for publication September 1, 1987

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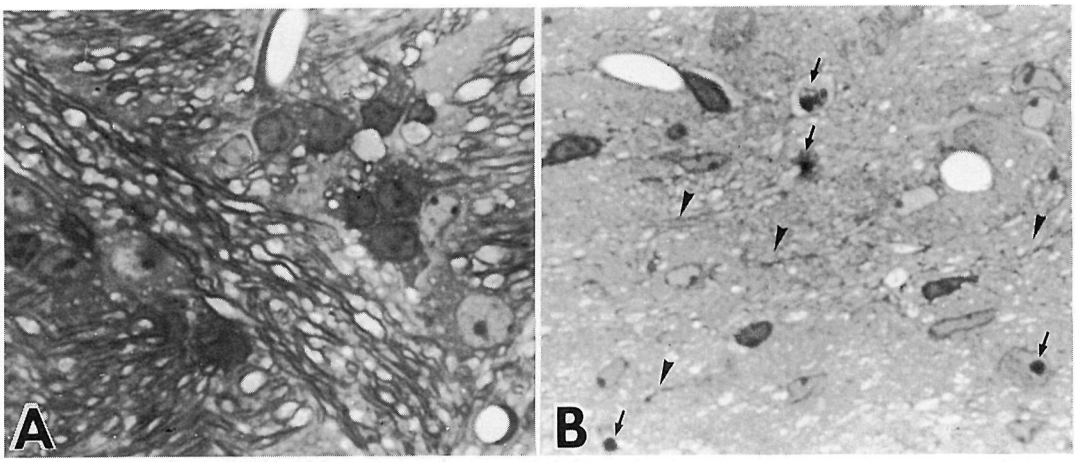

Fig. 1. Corpus callosum in a control (A) and in a Jimpy mouse (B) on postnatal day 21, stained with toluidine blue. In the control, various types of glial cells are among well-myelinated axons. Note the defective myelin sheaths in the white matter of a Jimpy mouse. Degenerating cells with a pyknotic nucleus (arrows) are scattering among naked axons. Arrow heads indicate a few myelinated axons in Jimpy corpus callosum. (×330)

phages with Jimpy specific cytoplasmic compartments (JSCC), and e) appearance of small lyphocytes. We paid particular attention to the appearance of hematogenous cells such as small lymphocytes and macrophages in the brain parenchyma without any vascular injury in the brain parenchyma. Some of them displayed contact with an oligodendrocyte, showing interdigitation between their surfaces. We supposed that the appearance of hematogenous cells might relate to the degeneration of the oligodendrocytes. Therefore, in the previous report (Imamoto, 1986), we speculated that the myelin deficiency might have been induced by the autoimmune response against oligodendrocytes producing myelin precursors. However, at least some of the oligodendrocytes survived in the white matter and formed a small number of myelin sheaths.

In the present experiment, we focused our attention on the fine structure of oligodendrocytes and aberrant myelin sheaths observed in the white matter of Jimpy mutant mice at various ages from postnatal day 3 to 25.

Results

The regions observed in this study are composed of the corpus callosum, capsula interna, cerebral peduncles, cerebellar medulla and its peduncles. The morphologial changes in the white matter of Jimpy mice were almost identical in each region but with some slight differences in the time course. In the controls, a small number of thin myelinated axons were already seen in the medulla oblongata during the neonatal period. The myelination in the cerebellar medulla started on postnatal day 3 and in the corpus callosum on postnatal day 7. Oligodendrocytes derived from glioblasts continuously increased in number dur-

ing the early postnatal period and progressively acquired darker cytoplasm corresponding to the subtypes of oligodendrocytes in the rats (Mori & Leblond, 1970; Imamoto et al., 1978). The sequential changes from LO to MO and finally to DO in an oligodendrocyte cell line corresponded well with the myelin formation in the mice as well as in rats. The myelination generally reached the abult mouse level by postnatal day 21 (Fig. 1A). Thus, the white matter of the abult mice exhibited a distribution of numerous MO and DO among well-myelinated axons under normal condition.

On the other hand, the number of oligodendrocytes in Jimpy white matter did not increase although the astrocytes revealed hypertrophy and hyperplasia. During the early period, there were many glioblasts capable of proliferation, and they gave rise to a moderate number of immature oligodendrocytes. Thereafter, however, sequential cell transformation rarely occurred and most of the young oligodendrocytes degenerated prior to cell maturation. Only a few LO were able to survive long enough to form myelin sheaths. The mature types of oligodendrocytes, such as MO and DO, were rarely found in Jimpy white matter even by postnatal day 25.

A few LO remaining in the white matter indicated a well-developed cytoplasm including intact cell organelles. At the periphery of such an oligodendrocytal perikaryon, however, membrane whorls or membranous lamellar bodies occasionally appeared without any relation to an axon syringe (Fig. 2). In the Jimpy corpus callosum on postnatal day 21, only a few myelinated axons

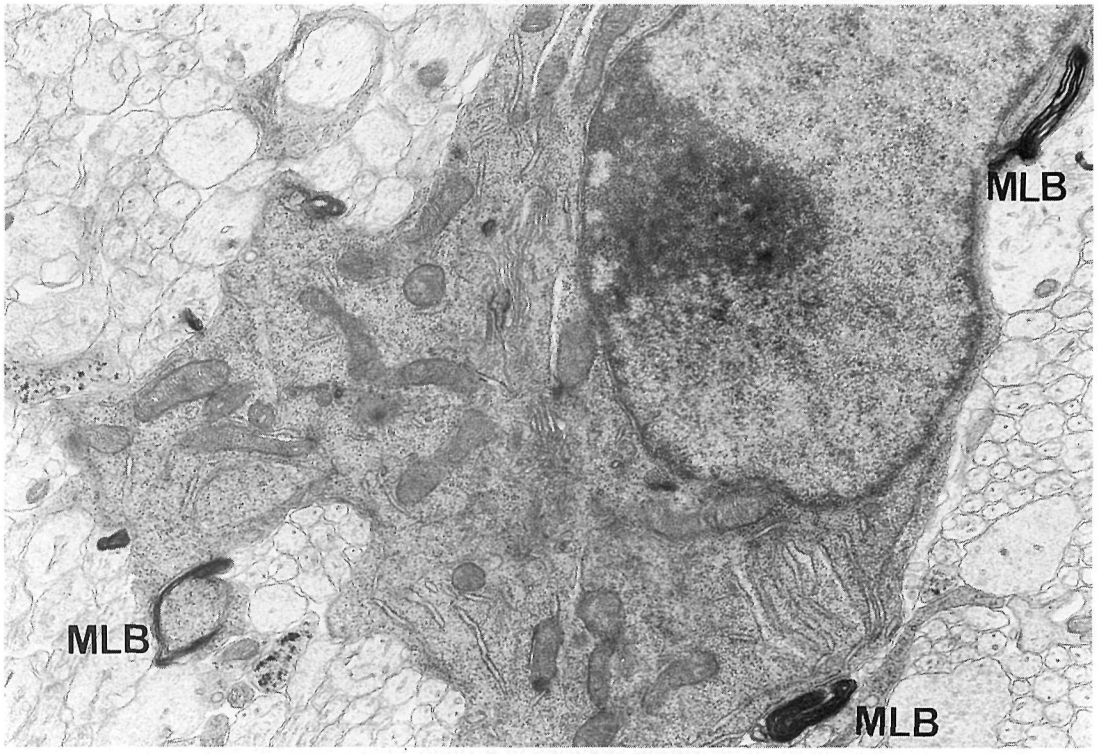

Fig. 2. Oligodendrocyte in the corpus callosum of a Jimpy mouse on postnatal day 18. Cell organelles are well preserved in the cytoplasm but small membranous lamellar bodies (MLB) are seen at the periphery of the perikaryon. (×18,000)

with thin lamellae were intermingled among naked axons. However, the total number of myelinated axons observed in the white matter varied considerably among Jimpy mice (Fig. 1B).

Oligodendrocytes remaining in the white matter extended their processes to surround axons and formed slightly aberrant myelin sheaths. The initial myelin sheath was formed by protoplasmic processes loosely wrapping the axons. These processes exhibited rich cytoplasm filled with microtubules and fine filamentous, and occasionally mitochondria (Fig. 3, 4). These structures the immature pattern of myelin sheaths which appeared during the early myelination period. Occasionally, partial membrane fusion was observed in the outer layers rather than in the inner layers, although vacuolations were often seen among the lamellae. Typical compacted myelin sheaths were rarely noted. Aberrant myelin sheaths appearing in Jimpy white matter were frequently composed of thin lamellae of uneven thickness and irregular periodicity, and cytoplasmic processes (Fig. 3, 4, 5). The terminal loop, or inner tongue of innermost lamellae often appeared larger than that in normal mice. The breakdown or vesicular dissolution of myelin sheaths was rarely observed during this experiment.

Degenerating cells with a homgeneous pyknotic nucleus in the vacuolated cytoplasm were often observed among naked axon (Fig. 6, 7). Under a light microscope, such pyknotic nuclei

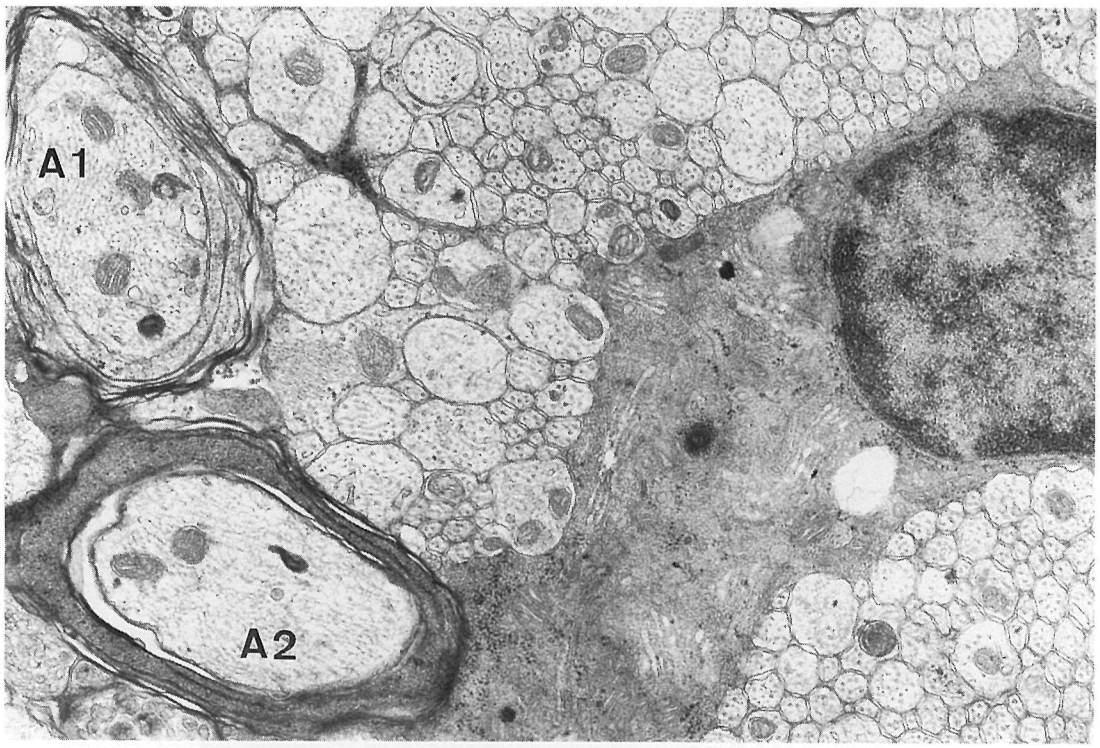

Fig. 3. Oligodendrocyte in the corpus callosum of a Jimpy mouse on postnatal day 17. The cell organelles are hazy in the vacuolated cytoplasm. The axons (A1, A2) are surrounded by thin cytoplasmic processes of oligodendrocytes. A typical periodicity can rarely be seen in such aberrant myelin sheaths. The dense oligodendrocytal processes around A1 indicate some degeneration. (×23,000)

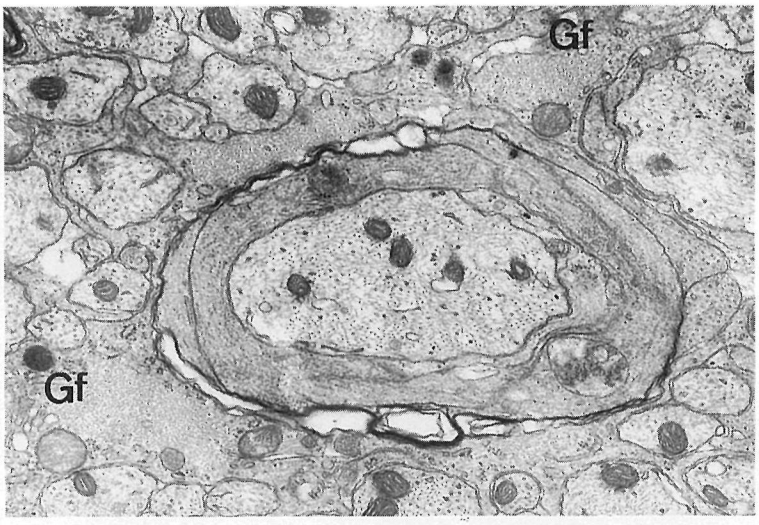

Fig. 4. Aberrant myelin sheath observed in the cerebellar medulla of a Jimpy mouse on postnatal day 18. The lamellar structure of myelin sheath is incompletely formed, showing a poor compaction of the cytoplasmic processes in the innermost layers. Vacuorations are seen in the outer lamellae. Astrocytal processes including numerous gliofilaments (Gf) extend among naked axons. (×15,000)

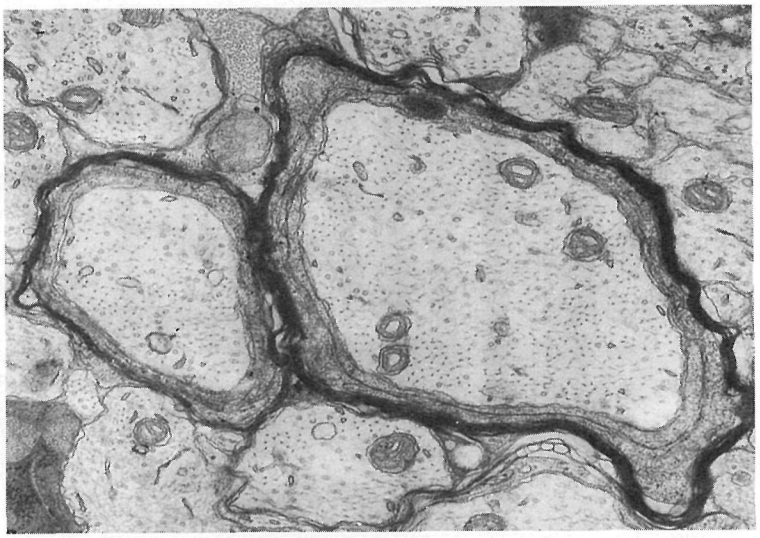

Fig. 5. Aberrant myelin sheaths observed in the cerebellar of a Jimpy mouse on postnatal day 19. The compaction of lamellae occurs in the outermost layers of the cytoplasmic processes surrounding axons. (×37,000)

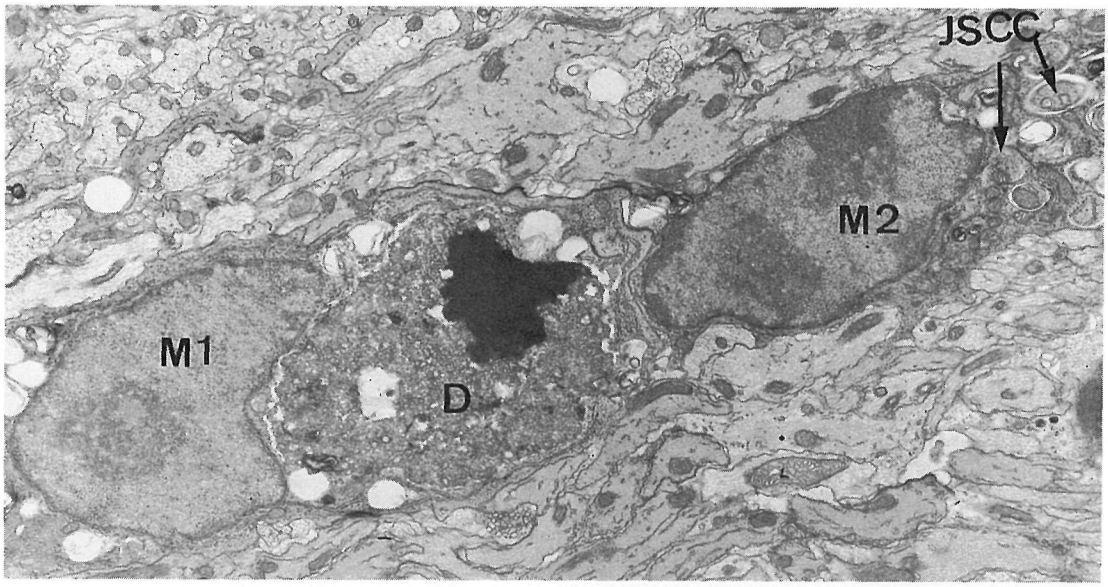

Fig. 6. Degenerating cell (D) in the cerebellar medulla of a Jimpy mouse on postnatal day 22. It shows a dense pyknotic nucleus in the vacuolated hazy cytoplasm and two macrophages (M1, M2) showing numerous vacuoles and JSCC in the cytoplasm. (×9,000)

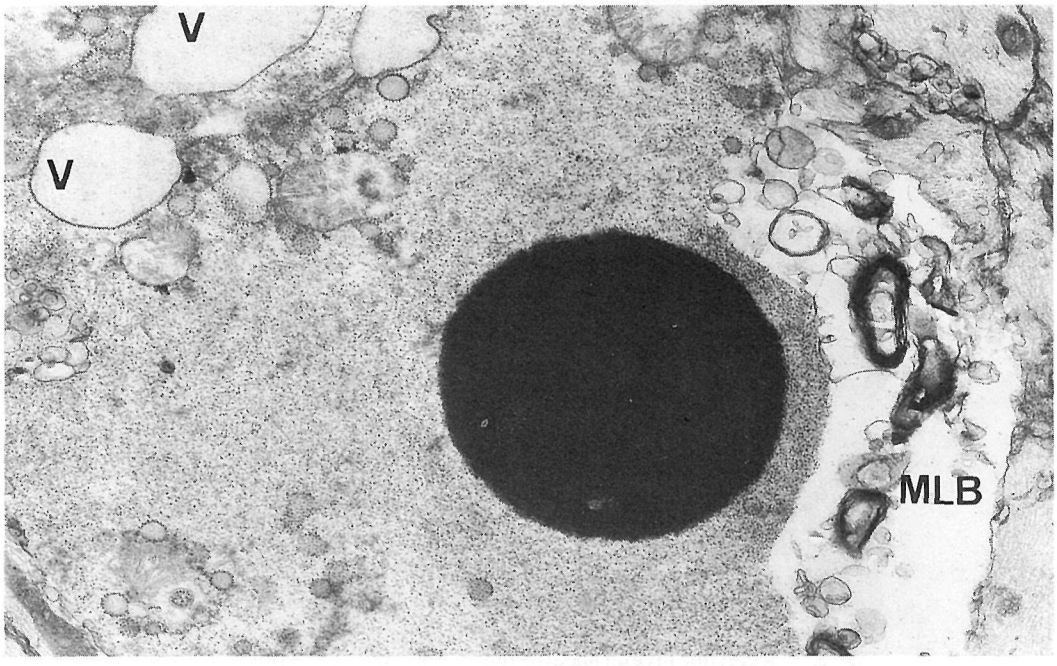

Fig. 7. Degenerating cell in the corpus callosum of a Jimpy mouse on postnatal day 12. It has a dense pyknotic nucleus in a necrotic hazy cytoplasm including fine ribosomal particles, swollen vesicles (V) and membranous lamellar bodies (MLB). (×15,000)

appeared as basophilic granular bodies of 1-5μm in diameter, when deeply stained with basic dyes such as toluidine blue, cresyl violet or nuclear fast red (Fig. 1B). These basophilic granular bodies surrounded by a hazy cytoplasm were more often observed in the Jimpy white matter than in the controls. In a test we counted the number of basophilic granular bodies appearing in the white matter of the Jimpy mice and noted that they were three times more numerous as compared with the controls. It was clear that the defect in maturation of oligodendrocytes resulted in the myelin deficiency in the Jimpy mice. Even though the degeneration of neurons and glial cells during normal brain development was generally seen during the early postnatal period, the degeneration of oligodendrocytes in the Jimpy mice continued through out the animal's lives. Thus, the total number of glial cells decreased tremendously, although the astrocytes increased in number. Most of the naked axons were surrounded by well developed astrocytal processes including numerous gliofilament bundles and glycogen particles, in stead of the normal myelin sheaths (Fig. 4).

In addition to the above, lipid-laden cells probably derived from macrophagic ameboid cells were found to increase in number considerably. The distribution of macrophagic ameboid cells reported in neonatal rats (Imamoto, 1981) was found to be the same in both the Jimpy mice and the controls during the neonatal period. Some macrophagic cells probably derived from such were observed to contain JSCC of various sizes in Jimpy mice, as described in the previous report (Imamoto, 1986). However, the origin of JSCC is still uncertain.

Discussion

Most of the axons in the Jimpy white matter were naked but some were surrounded by aberrant sheaths, revealing the absence of typical periodicity of the lamellae although some thin cytoplasmic processes of oligodendrocytes could be observed.

The present observation confirms that the myelin defect was caused by agenesis due to the numerical reduction of oligodendrocytes rather than the breakdown of once-formed myelin sheaths (Farkas-Bargeton, et al., 1972; Privat et al., 1972; Meier & Bischoff, 1975; Imamoto, 1986). In fact, the lamellar stacks or myelin remnants observed in the cases of demyelination in experimental allergic encepharomyelitis (EAE) produced by inoculation of myelin basic protein (MBP), were never found in Jimpy mice (Lassmann & Wisniewski, 1979; Raine et al., 1980). Some authors consider that impaired myelination in Jimpy mice may be due to a genetically determined disturbance in the glial differentiation, particularly in the spongioblasts (Kraus-Ruppert et al., 1973). However, given the fact that a few oligodendrocytes differentiated enough to form myelin sheaths, primary damage to oligodendrocytes is doubtful. In Quaking and Shiverer mice, recessive autosomal mutants, similar abnormalities were reported in the myelin structures, i. e. uncompacted myelin sheaths with many cytoplasmic islands among the lamellae (Wisniewski & Morell, 1971; Watanabe & Bingle, 1972; Privat et al., 1979; Inoue et al., 1981; Nagara & Suzuki, 1981). However, agenesis of myelin sheaths and dystrophy of oligodendrocytes in the central nervous system seemed severer in the case of Jimpy mice as compared with Shiverer and Quaking mice. On the other hand, the myelination in the PNS was very little affected in all three kinds of mutants (Rosenbluth, 1980; Farkas-Bargeton, 1972).

The morphogenesis of the myelin sheath is a temporarily ordered course initiating ensheathment of the axon by a single wrapping of the oligodendrocyte cell membrane, proceeding to the formation of multiple loose wrappings, and even-

tually fusing to form the mature and multilamellar myelin sheath (Raine, 1977). Thus, the aberrant myelin sheaths in Jimpy mice resemble the features observed during the early stage in the general course of myelination in the control mice. The fusion of inner leaflets of the unit membrane to form the major dense line seemed to be delayed in such myelin forming processes in Jimpy mice.

It is generally said that the myelin basic protein (MBP) is located on the innersurfaces of the cell membrane which has fused to form the major dense line of the myelin lamellae (Raine, 1977). MBP might serve as "structural cement" to maintain the compact structure of the sheath (Kies et al., 1965; Carnegie and Dunkley, 1975; Matthieu et al., 1980). Therefore, the reduction of MBP affects the compaction of the oligodendrocyte processes in wrapping axons (Inoue et al., 1981).

In the EAE induced by inoculation of myelin components, it is said that EAE immunoglobuline is bound by the intraperiod line of myelin (Johnson et al., 1979), and that invading macrophages may destroy the antigen-containing regions of the cell membrane of the oligodendrocytes (Raine et al. 1980; Epstein et al. 1983). The above authors also mentioned that when heated EAE serum was added to the culture medium it activated oligodendrocytes to form numerous processeâ and aberrant myelin sheaths. The wide spaced configuration of the myelin elaborated by the oligodendrocytes was caused by the membrane changes due to the binding of EAE immunoglobulin. Sandru et al. (1980) showed with immunocytochemistry of cultivated Jimpy brains that the maturation of oligodendrocytes was the cells were reactive for galactocerebrosides, but did not proceed further to the stage where myelin basic protein is normally detected. Up to the present date, the antigenicity of myelin components and oligodendrocytes have been revealed by several approaches.

We supposed that the myelination might apart at much later period in Jimpy mice than in the control ones because most of the oligodendrocytes differentiated at the early postnatal period, declined untill cell death occurred. The findings corresponded well to the results obtained by quantitative investigation suggesting a delay in the differentiation of various glial cells (Kraus-Ruppert et al., 1973; Imamoto, 1985) and protracted proliferation of glial cells from the glioblasts in the subependyma (Privat et al., 1982). It is probable that damage to oligodendrocytes disturbs their further development and survival in Jimpy mice. However, defective oligodendrocyte maturation may not be the principal damage to oligodendrocytes, because some of them can survive long enough to form myelin sheaths. The principal defect in the myelin deficiency is not yet known.

We assumed that oligodendrocytes can survive long enough to form myelin sheaths if they differencitate after than the period of the establishment of the blood brain barrier (BBB), because the presence of the BBB may somehow prevent antibodies and immunoeffective cells from entering the brain parenchyma. Thus, oligodendrocytes may not be affected completely by an autoimmune response in the late postnatal period.

Further experiments are required to determine whether the defect is located in the genetically determined intrinsic factor of oligodendrocytes themselves or rather is caused by exogenus factors affecting oligodendorcyte development in the CNS.

Acknowledgements

The authors are grateful to Mrs.A.Yamaguchi for her excellent secretarial assistance. The manuscript was kindly read by Mr. Henry Wyld in Paris.

References

1. Dunkley,P.R. & Carnegie,P.R. (1975) Amino acid sequence of the smaller basic protein from rat brain myelin. Biochem. J. 141, 243-55.
2. Epstein,L.G., Prineas, J.W. & Raine,C.S. (1983) Attachment of myelin to coated pits on macrophages in experimental allergic encephalomyelitis. J. Neurol. Sci. 61, 341-348.
3. Farkas-Bargeton,E., Robain,O. & Mandel,P. (1972) Abnormal glial maturation in the white matter in Jimpy mice. Acta Neuropathol. (Berl.) 21, 272-281.
4. Imamoto,K. (1981) Origin of microglia: cell transformation from blood monocytes into macrophagic ameboid cells and microglia. In "Glial and Neuronal Cell Biology" ed. by E.A. Vidrio & S.Fedoroff. 125-139.
   Alan R, Liss, Snc., New York.
5. Imamoto,K. (1985) Astrocytal changes in the white matter of Jimpy mice: Immunohistochemistry using antisera to glial fibrillary acidic protein. Arch. Histol. Jpn. 48, 411-419.
6. Imamoto,K. (1986) Appearance of hematogenous cells in the white matter of myeline-deficient Jimpy mice. Arch. Histol. Jpn. 49, 297-307.
7. Imamoto,K., Paterson,J.A. & Leblond,C.P. (1978) Radioautographic investigation of gliogenesis in the corpus callosum of young rats. 1. Sequential changes in oligodendrocytes. J. Comp. Neurol. 180, 115-138.
8. Inoue,Y., Nakamura,K. Mikoshiba,K. & Tsukada,Y. (1981) Fine structure of the central myelin sheath in the myelin deficient mutant Shiverer mouse, with special reference to the pattern of myelin formation by oligodendroglia. Brain Res. 219, 85-94.
9. Johnson,A.B., Raine,C.S. & Bornstein,M.B. (1979) Experimental allergic encephalomelitis: Serum immunogloblin binds to myelin and oligodendrocytes in cultured tissue. Ultrastructural-immunoperoxidase. Lab. Invest. 40, 568-575.
10. Kies,M.W., Thompson,E.B. & Alvord,E.C.Jr. (1965) The relationship of myelin proteins to exprimental allergic encephalomyelitis. Ann. New York Acad. Sci. 122, 148-160.
11. Kraus-Ruppert,R., Herschkowitz,N. & Furst, S. (1973) Morphological studies on neuroglial cells in the corpus callosum of the Jimpy mutant mice. J.Neuropath. Exp. Neurol. 32, 197-202.
12. Lassmann,H. & Wisniewski,H.M. (1979) Chronic relapsing experimental allergic encephalomyelitis: Morphological sequence of myelin degradation. Brain Res. 169, 357-368.
13. Matthieu,J.-M., Ginalski,H., Friede,R.I., Cohn,S.R. & Doolittle,D.P. (1980) Absence of myelin basic protein and major dense line in CNS myelin of the mld mutant mouse. Brain Res. 191, 278-283.
14. Meier,C. & Bischoff,A. (1975) Oligodendroglial cell development in Jimpy mice and controls. An electron-microscopic study in the optic nerve. J.Neurol. Sci., 26, 517-528.
15. Mori,S. & Leblond, C.P. (1970) Electron microscopic identification of three oligodendrocytes and a preliminary study of their proliferative activity in the corpus callosum of young rats. J. Comp. Neurol. 139, 1-30.
16. Nagara,H. & Suzuki,N. (1981) Chronological study of oligodendroglial alterations and myelination in Quaking mice. Neuropath. Appl. Neurobiol. 7, 135-149.
17. Privat,A., Jacque,C., Bourre,J.M., Dupoafy,P. & Baumann,N. (1979) Absence of the major dense line in myelin of the mutant mouse "shiverer" Neuroscience Lett. 12, 107-112.
18. Privat,A., Robain,O. & Mandel,P. (1972) Aspects ultrastructuraux du corpus calleux chez la souris Jimpy. Acta Neuropathol. (Berl.) 21, 282-295.
19. Privat,A., Valat,J., Lachapelle,F., Baumann,

N. & Fulcrand,J. (1982) Radioautographic evidence for the protracted proliferation of glial cells in the central nervous system of Jimpy mice. Develop.Brain Res. 2, 422-416.
20. Raine,C.S. (1977) Morphological aspects of myelin and myelination. In "Myelin", ed, by P.Morell. Plenum Press, New York. 1-49.
21. Raine,C.S., Barnett,L.B., Brown,A., Behar,T. & CcFarlin,D.E (1980) Neuropatology of experimental allergic encephlomylitis in inbred strains of mice. Lab. Invest. 43, 150-157.
22. Rosenbluth,J. (1980) Cenral myelin in the mouse mutant Shiverer. J. Comp. Neurol. 194, 639-648.
23. Sandru, L., Siegrist, H.P., Wiesmann, U.N. & Herschkowitz, N. (1980) Development of oligodendrocytes in Jimpy brain cultures. In "Neurological Mutation Affecting Myelination" ed. by N. Baumann. 469-475. Elsevier/North-Holland Biomedical Press.
24. Watanabe,I. & Bingle,G.J. (1972) Dysmyelination in "Quaking" mouse Electron microscopic study J. Neuropathol. Exp. Neurol. 31, 352-369.
25. Wisniewsky,H. & Morell,P. (1971) Quaking mouse: Ultrastructural evidence for arrest of myelinogenesis. Brain Res. 29, 63-73.

髄鞘欠損を示すJimpyマウスの脳内白質部におけるオリゴデンドロサイトの微細構造

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髄鞘欠損を示す突然変異種Jimpyマウスは，X染色体上の劣性因子jpにより伴性遺伝してゆき，約25日の寿命で死亡する。この間の脳内白質部に残存するオリゴデンドロサイトを電顕的に観察した．

正常雄マウスでは，髄鞘形成は生後3日に小脳髄質部で，やや遅れて7日に脳梁部で始まり，生後21日でほぼ成体のレベルに達す．

一方Jimpyマウスでは，グリオブラストから幼弱なオリゴデンドロサイトへの分化はあるが，その後，髄鞘形成にともなって進行するオリゴデンドロサイトの一連の成熟過程（LO-MO-DO）が生じず，多くの細胞はLOになる前後で変性消失し，MOやDOは認められかった．変性細胞は，生後12日頃に最も多く，その数は正常マウスの3倍以上にも達していた．それらは，1-5μmの均一な高電子密度の核を持ち，突起を失って球状になりリボゾーム様粒子や膨化した小胞の散在する細胞質で包まれていた．

残存する少数のオリゴデンドロサイトは，微細繊維や微小管を含む突起をのばして，軸索を一重ないし数重に取り巻き，時には髄鞘形成を示すものもあった．しかし，周期線や周期間線などの典型的層板構造を示すものは稀で，多くのものは膜の融合のない細胞質突起の状態のままであった．